FORMULATION OF LIPID AND LIQUID NANOPARTICLES OF SEEDS EXTRACT OF *Pongamia pinnata* AS ANTCANCER AGENT

Proposed work will be done:

a) Literature survey.
b) Plant identification and collection from the various wild and cultivated sources.
c) Ethanolic extraction of plant seeds of *Pongamia pinnata*.
d) Phytochemical screening.
e) Preparation of nanoparticles of phytochemicals with the help of available metals.
f) Acute toxicity study.
g) Bio-distribution and Pharmacokinetic studies.
h) Cytotoxic activity by *in-vitro* studies using established human tumor cell lines.
i) Pharmacological studies: *In-vivo* anticancer study by using Ehrlich Ascites Carcinoma cells. Following parameter required to be done:
    1. Tumor mass and packed cell volume.
    2. Percentage increase in life span and mean survival time
    3. Hematological parameters
    4. Serum biochemical parameters
    5. Enzymatic antioxidant parameters
j) Comparative studies: Comparative studies would be done to check the efficiency of each type of nanoparticle before going on further studies.
k) Statistical analysis
l) Result and discussion

Materials and Methods

Cell lines

Colon carcinoma, hepatocarcinoma, cervical carcinoma, murine macrophage cell lines etc. (depends on availability) cell lines will collect from the available resources at the time of study.

Collection and Extraction of Plants

For the present study, the seeds of plant *Pongamia pinnata* (Leguminoseae) will be collected. The plant species will be scientifically authenticated in the National Herbarium and Plant Laboratory, Delhi. Preparation of Extract will be achieved by extracting the powdered seeds of the plants with ethanol using Soxhlet's apparatus.

Qualitative phytochemical screening

The qualitative phytochemical analysis of Alkaloids, Saponin, sterol, glycoside, tannin, Terpenoid, Protein etc. would be performed as the procedure followed by the previous investigators.

Determination of total flavonoid content (TFC)

Aluminium chloride ($AlCl_3$) colorimetric assay will be performed for the determination of total flavonoid content (TFC). The absorbance of the reaction mixture will be measured with the help of a UV-Visible spectrophotometer.

Determination of total phenolic content (TPC)

Folin-Ciocalteu's technique will use to determine total phenolic content (TPC). The absorbance will be measured using a UV-Visible spectrophotometer.

Green synthesis and chemical characterization of AuNPs

The extract of the seeds of *Pongamia pinnata* obtained for the green synthesis method will be extracted. With the help of HAuCl4.H2O, the gold nanoparticles will be prepared for the study.

Different analytical techniques will use for the characterization of Au nanoparticles:

1) TEM analysis of the Au nanoparticles will be performed.

2) In the UV Vis spectroscopy analysis, characteristic absorption bands of Au metal will be examined before and after the nanoparticles synthesis process.

3) The biomolecules related to the Au nanoparticles reduction including secondary metabolites will be detected by the FT-IR.

Synthesis of Cu nanoparticles

Copper nanoparticles are synthesized by plant extracts such as via the reduction of aqueous copper ions. The formation of an average size of 40 nm Cu nanoparticles will be confirmed by 578-nm peak at UV Visible spectrometer.

Synthesis of ZnO nanoparticles

ZnO (Zinc oxide) nanoparticles are of great interest due to inexpensive to synthesize, safe, and easy method of synthesis. These nanoparticles possess high exciton binding energy of 60 meV and a large band gap of 3.37 eV, and due to this, these show various semiconducting properties such as high catalytic activity, wound healing, anti-inflammatory, ultraviolet filtering properties and extensively used in various cosmetics such as sunscreen. These nanoparticles revealed various biomedical applications too such as antifungal, antibacterial, drug delivery, anti-diabetic, anticancer. Up to now, numerous works have been reported for ZnO synthesis and utilization by plants, microorganisms, and others. Plant parts like flower, root, seed, leaves, etc., are used for the synthesis of ZnO nanoparticles.

In-vitro DPPH and ABTS assay for the determination of antioxidant activities

Free radical scavenging will determine by 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) assay. The percentage of DPPH and ABTS scavenging will calculate by the following formula.

$$\%\text{Radical scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} * 100$$

Anticancer activities

Cell culture and growth assay

The anticancer activities of the plant extract will measure in different cell lines as available. Cell growth will be measured using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay.

Wound healing assay

Cell migration will analyze using a wound healing assay by using the standard protocol.

Acute oral toxicity study

Acute oral toxicity will be performed by following OECD guideline – 420. Fixed dose procedure for all types of nanoparticles will be found and it will also find that dose increasing up to 2000 mg/kg body wt. shown no toxicity or mortality in experimental rats. The LD_{50} of the methanol extract of the plants as per OECD guidelines – 420 is greater than 2000 mg/kg.

Model (1)

Experimental animals

Swiss albino mice having weight 25-30 gm will be kept in quarantine for 10 days under standard husbandry conditions (27±3^{0}C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and will be given standard food and water ad libitum.

Tumor cells

Ehrlich ascites carcinoma (EAC) cells will be obtained. EAC cells 9 days old will be used for the screening of the MMPs (Matrix metalloproteinases).

Following parameters will be used to evaluate Anti-tumor activity of the plant extracts

Bio-distribution/Organ Distribution

Bio-distribution will be carried out in mice as well as in Ehrlich Ascites Tumor bearing mice of 2-3 months age and weighing about 25g.

Percentage increase life span (% ILS)

The effect of test samples on tumor growth will be monitored by recording the mortality daily for a period of 9 days and percentage increase in life span (%ILS) will be calculated by following equation.

Increase in life span = T-C / C X 100 Body weight

Body weights of the experimental mice will be recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

Hematological parameters

Hematological parameters will be done with the help of cell analyzer. Parameters to be perform will be Hemoglobin, RBC, WBC, Basophils, Neutrophils, Eosinophil, MCV, MVCV etc.

Serum Biochemical Parameters

Estimation of serum biochemical parameters collected blood was centrifuged and serum was used for the estimation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) by using commercially available reagent kits. Serum total protein content was assessed by the method of Lowry et al. (1951).

Determination of tissue antioxidant parameters

The antioxidant parameters of processed liver and kidney tissues were assessed by measuring the level of lipid peroxidation (Ohkawa et al., 1979), the amount of reduced glutathione (Ellman, 1959), and the enzymatic activities of catalase (Sinha, 1972) and superoxide dismutase (Kakkar et al., 1984). The total protein content of liver and kidney tissue for these tissue biochemical parameters was determined by the method of Lowry et al. (1951).

Model (2)

Experimental Animals

Zebrafish strains would be obtained from the available sources. Fish will house in an automated fish housing system at 28^0C.

Compounds

The compounds would dissolve in DMSO and would be diluted for zebra fish or with DMEM for cell culture respectively.

Real-time RT-PCR assay

The real time RT-PCR would be done for the tumor growth in the animals. The primer sequences should be as follows:

p53:

50 -ATGTGGTGCCTGCCTCAGA-30 (sense)

50 -CTTCGTCCTTCACCATCAGCTT-30 (antisense)

Beta-actin:

50 -TGACAACGGCTCCGGTATG-30 (sense)

50 -TTCTGTCCCATGCCAACCAT-30 (antisense)

Analysis of apoptosis

Apoptotic cells will identify using the cell death detection kit. They will be then subjected to the TUNEL assay. The fluorescent signal will visualize and image should be taken.

***In-vitro* Anticancer Analysis**

Experiments will be performed using the available human cancer cell lines. The Sulforhodamine B (SRB) assay will be performed according to the method. The optical density will be read on an automated spectrophotometer plate reader at 540 nm. For cells growing in suspension (e.g., leukemia), the same method will be employed.

COMPARATIVE STUDIES

The comparative studies will perform among all kinds of nanoparticles in leu of their anticancer properties.

STATISTICAL ANALYSIS

Results of estimation of biochemical and functional parameters will report as mean value SEM. The variation in a set of data will estimate by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values may do by using Dunnet's test (Sigma stat 3.5). P values <0.05 will consider statistically significant.